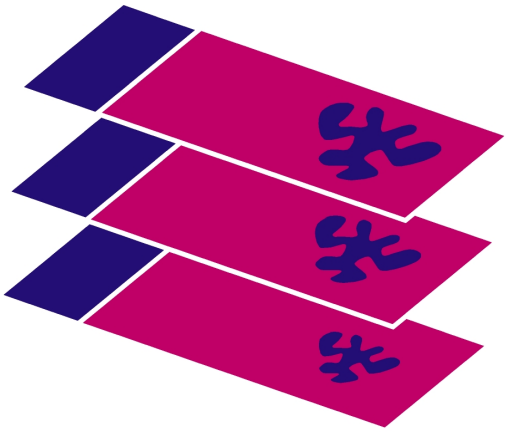

3DHISTECH

MembraneQuant Module 1.12

User's Guide

19 June 2009

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20070007642.2; US 2008/0306964 A1; HK07113109.4; EP05702806.0

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Font types and symbols

Example	Abbreviation or term that is explained in the section Terms and Abbreviations.
Example	Words or characters that appear on the screen. These include field names, screen titles, pushbuttons and menu names, paths or options. Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+O** key combination.
Example	Cross-references to other documents.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task or that special care should be taken when using certain feature of the device.
Important!	An important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
Tip!	A suggestion about using the application in some other way or to some interesting purpose.

Terms and abbreviations

Annotation	A particular detail of a digitized slide. MembraneQuant can perform measurements on annotations.
CCC	The Color Deconvolution setting is stored in a .ccc file.
CSV format	Comma separated value
Digital slide	Slide digitized with a MIRAX scanner microscope, for example MIRAX SCAN. Consists of an MRXS file and a folder with the same name as the MRXS file.
Field	In MembraneQuant: an annotation. In ImmunoScreener: a grid cell.
Layer	A group of cell compartments matching the same stain and size criteria.
Measurement	MembraneQuant can perform two types of measurements: • field-specific (related to the annotation) • object-specific
MISP	MIRAX Imaging Segmentation Profile A MembraneQuant segmentation and measuring algorithm is stored in a MISP file.
MRXS	MIRAX Slide File extension of a digitized MIRAX slide.
Object	A cell compartment (for example, membrane tissue), cell or tissue that is relevant for the immunohistochemical analysis.
Segmentation	Identification of cells or cell compartments based on their different reactions to immunohistochemical staining.

1 About MembraneQuant

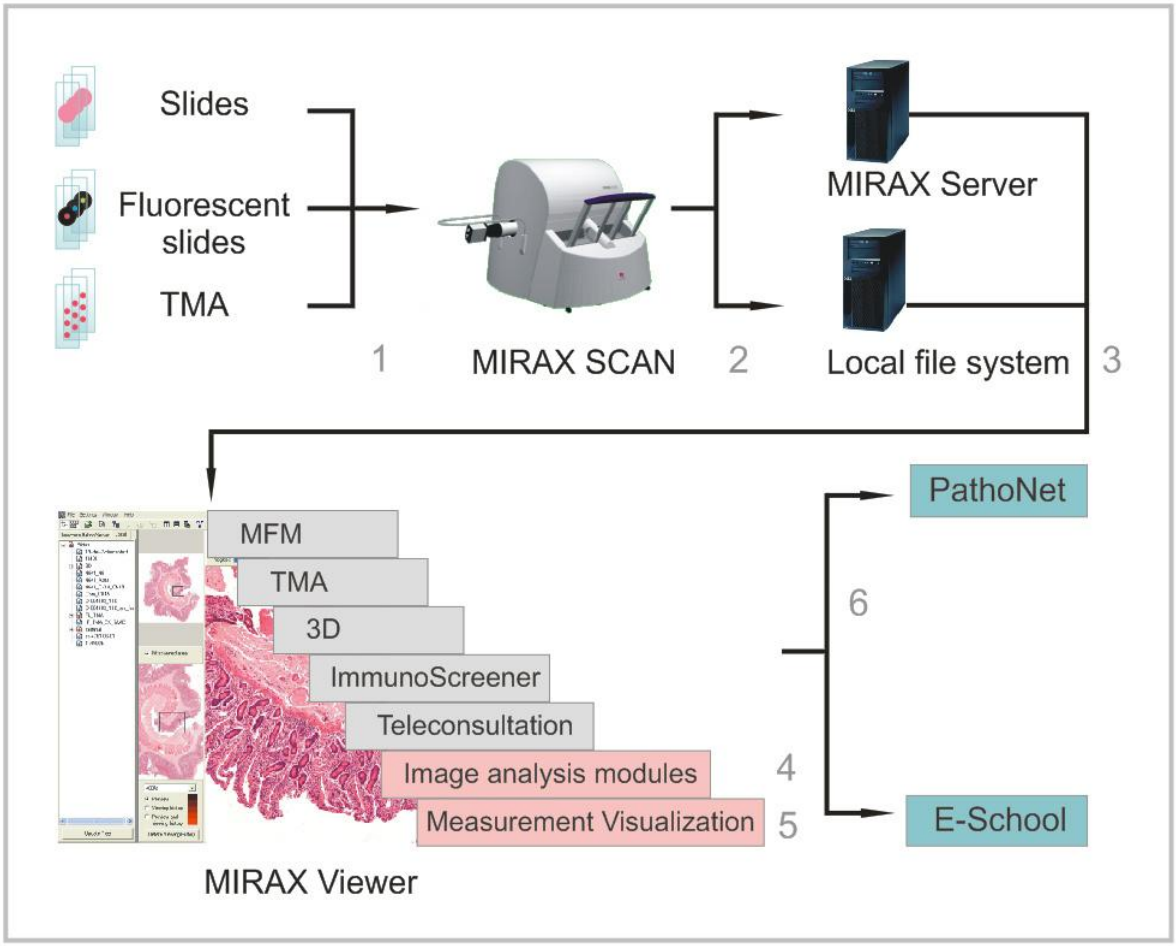
MembraneQuant is an optional module for MIRAX Viewer. It allows you to perform examinations of immunolabelled cell membrane on the virtual slides and digitalized with MIRAX microscopes.

- **MembraneQuant** performs *segmentation* and *measurement*s on MIRAX *digital slide*s based on the colors of the immunoreactive cell membrane.

- Based on the intensity of the immunohistochemical dyeing, it can classify the cell membrane.

1.1 MembraneQuant in the MIRAX product family

The following picture shows how **MembraneQuant** and **Measurement Visualization** extend MIRAX Viewer and how they fit into the MIRAX product family.



1. Slides are digitized with MIRAX microscopes, for example, MIRAX SCAN. For more details, see *MIRAX SCAN User's Guide*. A digitized slide consists of an *MRXS* file and a folder with the same name as the *MRXS* file.

2. Digitized slides are stored on a computer. You can also use a dedicated server (for example, MIRAX Server) for storing the MIRAX slides.

3. Slides digitized with a MIRAX microscope can be viewed in MIRAX Viewer. If the slide is stored on a MIRAX Server, it is opened through the Teleconsultation module. Local slides are opened directly with MIRAX Viewer.

4. **MembraneQuant** allows you to perform *measurement*s on *digital slide*s and to export *measurement*s in *CSV format*.

5. **Measurement Visualization** displays the **MembraneQuant** data as a scatter plot, a histogram, an image gallery or a classification gallery.

6. *Digital slide*s, including slides that store **MembraneQuant** *measurement*s, can be shared via PathoNet (www.pathonet.com). They can also be used in E-School as training and/or exam materials.

1.2 Overview of Membrane Analysis

You can perform two types of tasks in **MembraneQuant**:

- set algorithms then run them on a slide or a set of slides
- view the *measurement* results with the **Measurement Visualization** module, save the *measurement annotation*s with the slide or export the *measurement* results to *CSV* file(s)
- create a report based on your saved *measurement*s

1.2.1 MembraneQuant algorithms

MembraneQuant is a membrane analysis application with color deconvolution profile settings. You can set the parameters of smoothness and merger, you can also filter the areas by size. The information necessary for the algorithm is not saved on the slide but in a MIRAX Image Segmentation Profile (*MISP*) file, which can be used later to evaluate other *annotation*s on the same slide or on other slides.

Note: Since **MembraneQuant** enables you to make *measurement*s on the slides and digitalized image processing can cause loss of quality, please, always make sure that the *measurement*s are acceptable to you. *Measurement* values are optimized by default; set parameters manually only if the result is not satisfactory to you.

1.2.2 Annotations, Fields, and Objects in MembraneQuant

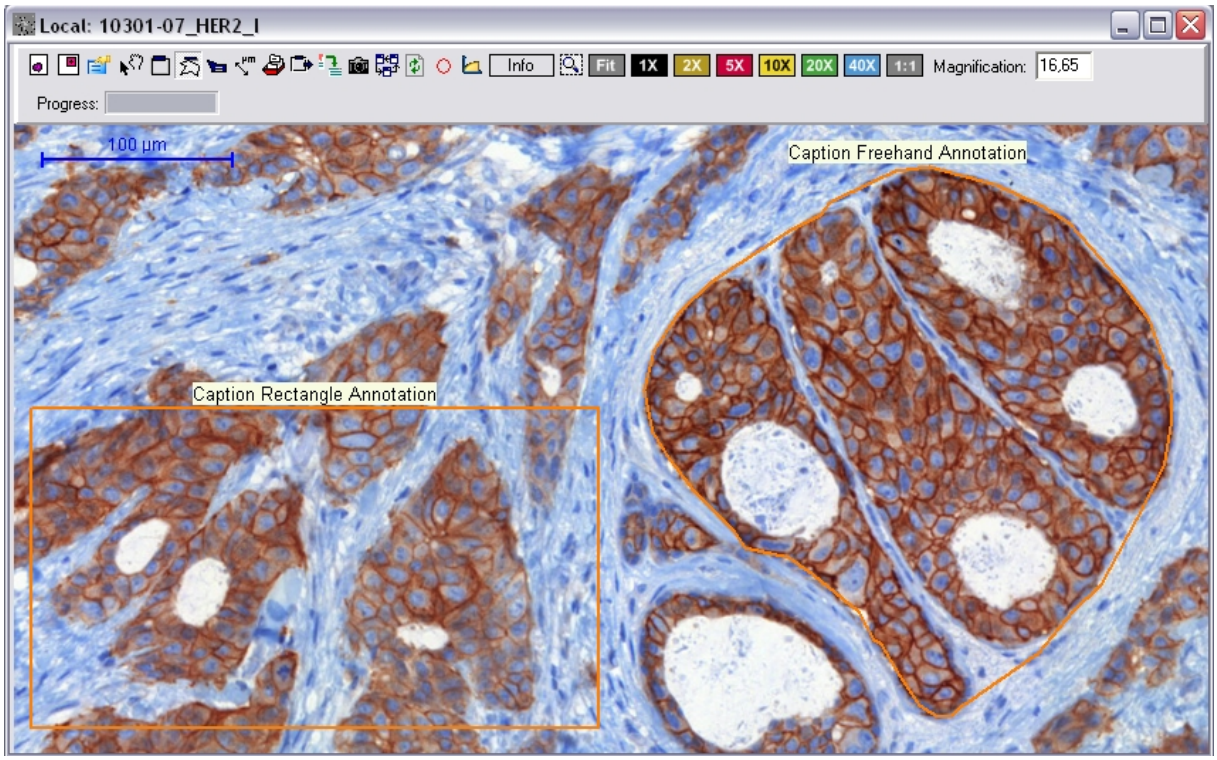
MembraneQuant uses *annotation*s. If you want to perform *measurement*s on the entire digitized area of the slide, you need an *annotation* that covers the entire area. You can use any of the *annotations* created in MIRAX Viewer, including *annotation*s containing **MembraneQuant** *measurement*s.

Tips!

You can add a caption and a description to an *annotation*. If you want to see a piece of information, for example a short comment, to be visible in the gallery that you can export from **MembraneQuant**, add it to the caption. Only the *annotation* caption is exported, the description is not.

Create unique captions to make *annotations* distinguishable, thus the *measurements* can be easily identifiable.

The following image shows a virtual slide treated with *IHC* stains. There are two *annotations* on the slide, these are possible targets for **MembraneQuant** analyses.



Annotation types:

(1) *field:* user-defined *annotation* (rectangle, polygonal/freehand)

(2) *object* (cell membrane): algorithm-defined *annotation* in the *field*

Note: A *layer* consists of *objects* (cell membranes) of a certain kind.

1.2.3 CCC files

MembraneQuant saves Color Deconvolution settings into a *CCC* file, that contains base settings by default.

1.2.4 MISP files

MembraneQuant algorithms are saved in *MISP* files that store the following information about the algorithm:

- *segmentation* algorithm
  Filters for noise reduction, *segmentation* and *object* separation so that **MembraneQuant** can isolate *object*s (for example, cell membranes) on the digitized slide.

- *measurement* parameters
  For example, the diameters (shorter/longer), the distance between two *object*s or the number of *object*s in an *annotation*.

1.2.5 Using a MISP file on other slides

You can reuse the *MISP* on other slides from the same batch (for example, on slides that are from the same specimen) without modifications. You can also use the same algorithm on slides from other specimens with the same staining.

You might need to adjust the profile to ensure that differences in the specimen or stain quality do not affect the result.

1.2.6 MembraneQuant Measurements

MembraneQuant creates the following types of *measurement*:

- *object-specific measurement* (related to an *object*)
  - is related to an *object*, for example, its area. For more information, see Appendix A1.

- *field-specific measurement* (related to an *annotation*)
  - sums up *object* data, for example the total area of *object*s of the same *layer* in an *annotation*. For more information, see Appendix A2.
  - conveys information about the *annotation*, for example its total area. For more information, see Appendix A2.

1.2.7 Measurement Export in CSV Format

You can export **MembraneQuant** *measurements* as *CSV* files. Both *field*-level and *object*-level *measurements* are exported.

You can choose between the following options:

<table>
<tr><td>Object-Specific CSV</td><td colspan="2">Saves the parameter values for each object in the annotations that you select for the CSV export.
If you use an existing file name, the old file will be overwritten.</td></tr>
<tr><td rowspan="3">Cumulative CSV</td><td>Single File Cumulative CSV</td><td>Multi File Cumulative CSV</td></tr>
<tr><td>• One cumulative file will be created</td><td>• One cumulative file will be created for each layer of objects detected</td></tr>
<tr><td colspan="2">• If you enter a new file name, the CSV export will contain all data in the project.
• If you select an existing CSV file, only the summary data will be added to the existing file. Use this option to create a cumulative file, for example for longitudinal studies or to follow-up a patient's history.</td></tr>
</table>

Tips!

Check the documentation of your statistical or spreadsheet application to ensure that the *CSV* file exported from **MembraneQuant** is compatible with that application.

Also, make sure that the regional settings of the operating system, MIRAX Viewer and the post-processing application are the same. Otherwise, separators (comma, semicolon, etc.) used during the *CSV* export will not be recognized in the statistical or spreadsheet application.

1.3 Overview of Measurement Visualization

Measurement Visualization is an extension to **MembraneQuant** which allows you to create a graphical representation of *measurement* results obtained from the *MembraneQuant algorithm* run on a slide (or set of slides grouped into an *ImmunoScreener project*). For more information on *ImmunoScreener*, see *ImmunoScreener Module for MIRAX Viewer 1.12 User's Guide.*

Measurement Visualization works only with *object*-level *measurement*s, which means that if you select three *annotation*s, all *object* data are summed up, but there is no information regarding how many data items derive, and from which *field*s.

2 Installing MembraneQuant and Measurement Visualization Bundled with MIRAX Viewer 1.12

MembraneQuant and **Measurement Visualization** are not stand-alone applications: you need MIRAX Viewer to run them. Therefore, you have to install MIRAX Viewer before installing the application.

2.1 Prerequisites for the MIRAX Viewer Installation

Prerequisites	Description
Hardware	**Minimum**: Pentium P4, 3GHz, 1GB RAM **Recommended**: Pentium C2D (DualCore), 2 GHz, 2GB RAM
Operating system	Microsoft Windows XP
Disk space	MIRAX Viewer 1.12 bundled with the **MembraneQuant** and **Measurement Visualization** modules 1.12 **Minimum**: 110 MB

2.1.1 Licensing

You receive a product-specific license when purchasing any of the 3DHISTECH applications.

2.2 Installing MIRAX Viewer and Its Modules

1. Launch the **3DHISTECH_Apps.exe** file that you received on a 3DHISTECH installation CD or downloaded in a compressed file.

2. Follow the instructions in the installation wizard.

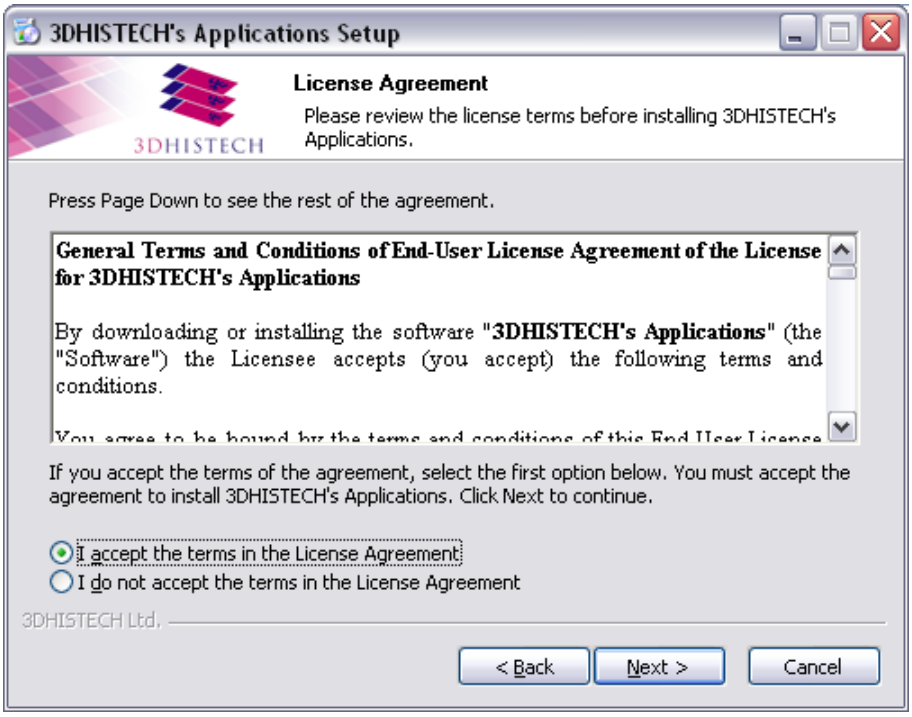
3DHISTECH's Applications Setup
Welcome to the Setup Wizard of 3DHISTECH's Applications
This wizard will guide you through the installation of 3DHISTECH's Applications.
It is recommended that you close all other applications before starting Setup. This will make it possible to update relevant system files without having to reboot your computer.
Click Next to continue.
Next >
Cancel

3. By default, the installation folder is the following:

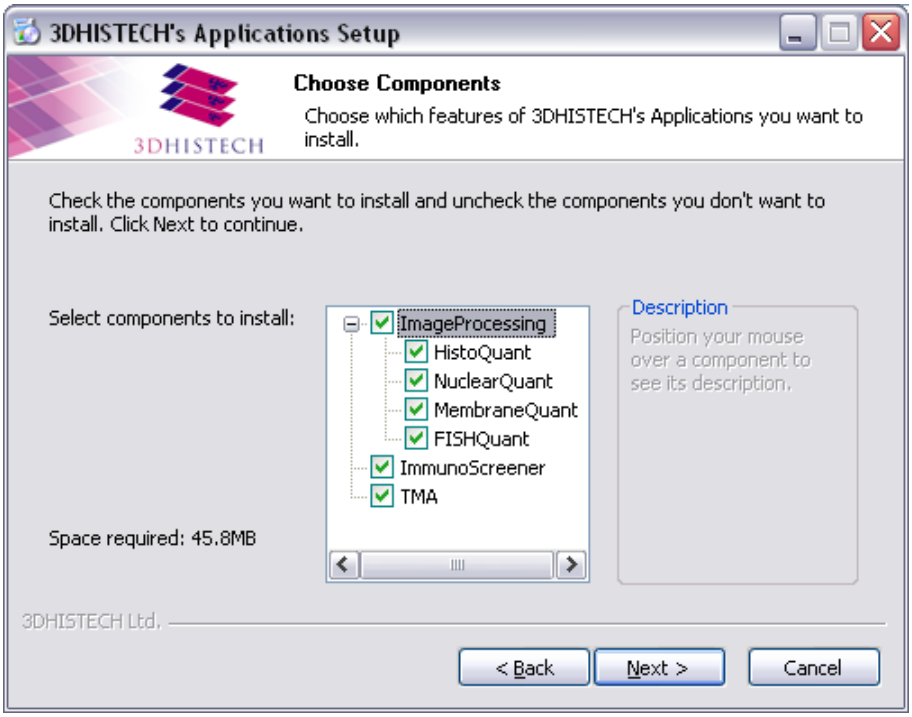
   C:\Program files\3DHISTECH\MIRAX Viewer

4. Set the license mode. If necessary, select install with HASP dongle, otherwise select a license file.

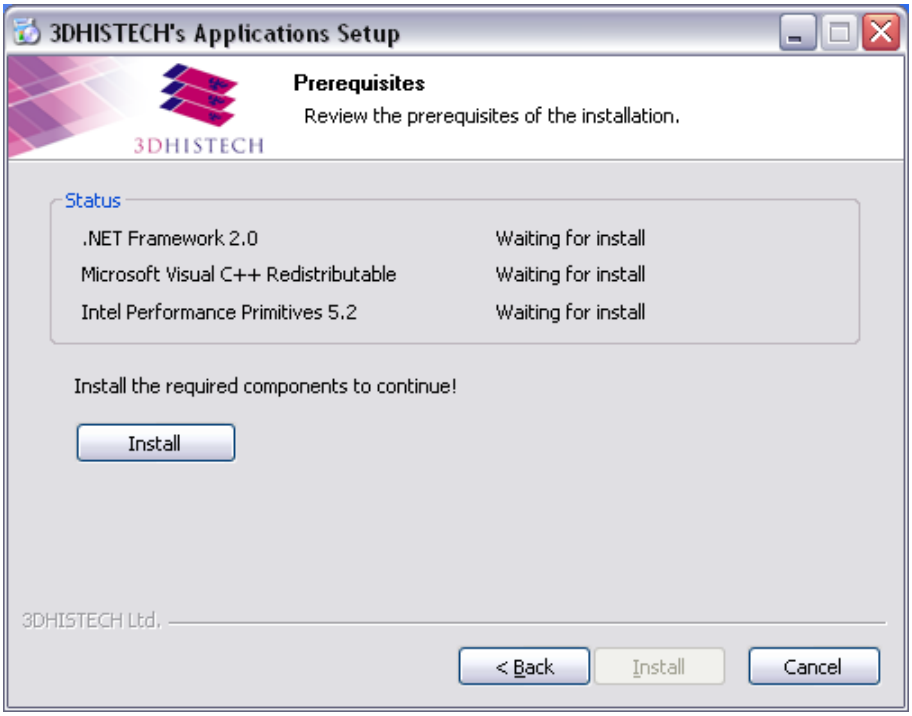
5. Accept the End-User License Agreement, and click **Next**.


3DHISTECH's Applications Setup
License Agreement
Please review the license terms before installing 3DHISTECH's Applications.
3DHISTECH
Press Page Down to see the rest of the agreement.
General Terms and Conditions of End-User License Agreement of the License for 3DHISTECH's Applications
By downloading or installing the software "3DHISTECH's Applications" (the "Software") the Licensee accepts (you accept) the following terms and conditions.
If you accept the terms of the agreement, select the first option below. You must accept the agreement to install 3DHISTECH's Applications. Click Next to continue.
I accept the terms in the License Agreement
I do not accept the terms in the License Agreement
3DHISTECH Ltd.
< Back
Next >
Cancel

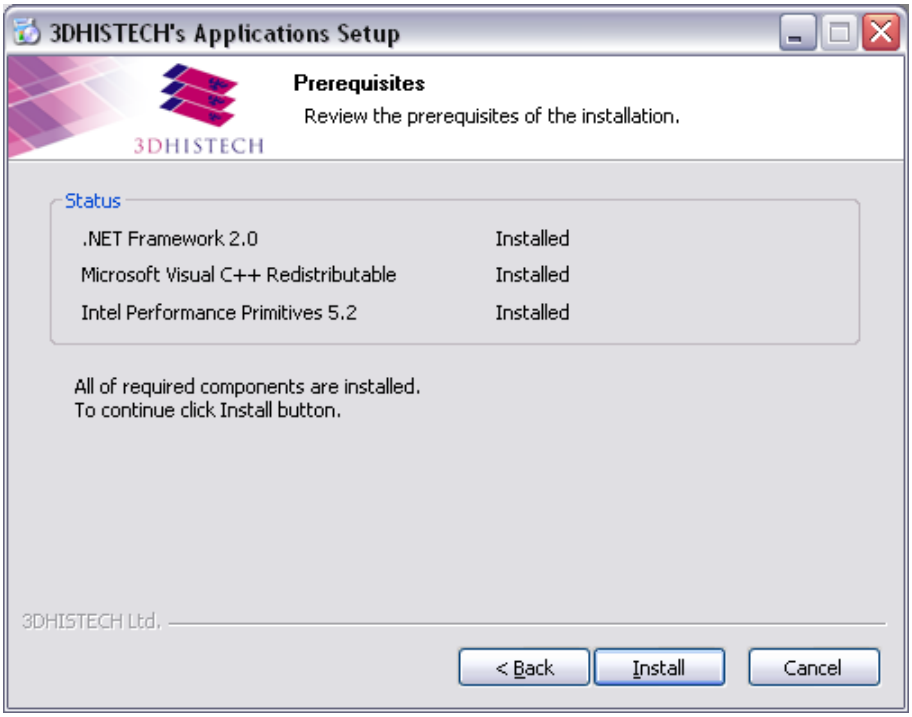
6. Select the components you want to install.



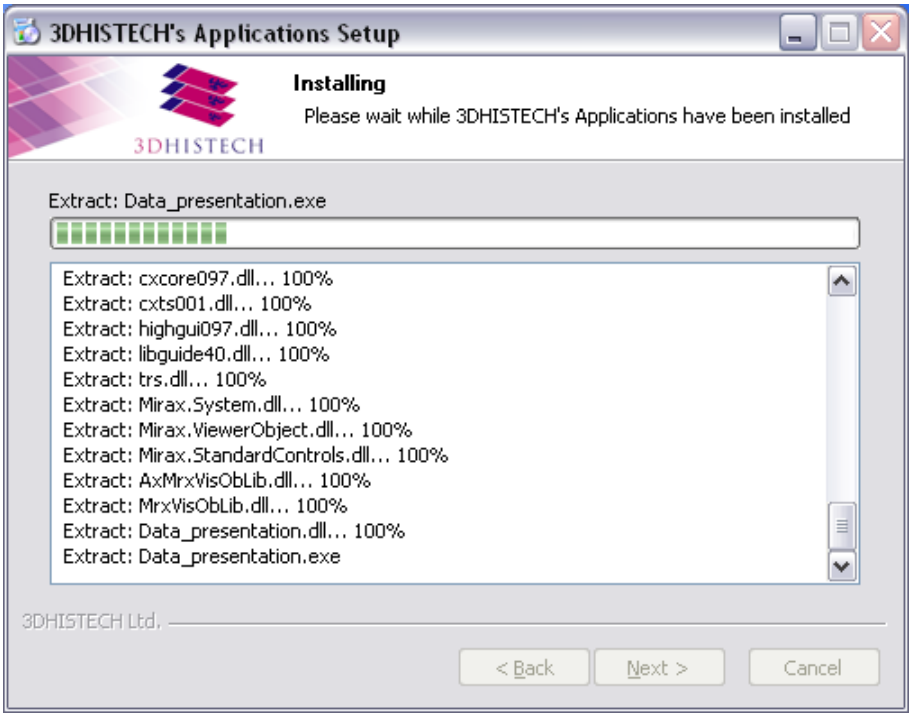
7. If not previously done, you have to install the prerequisites of the installation.



8. Click **Install** to continue with the installation of the selected components.

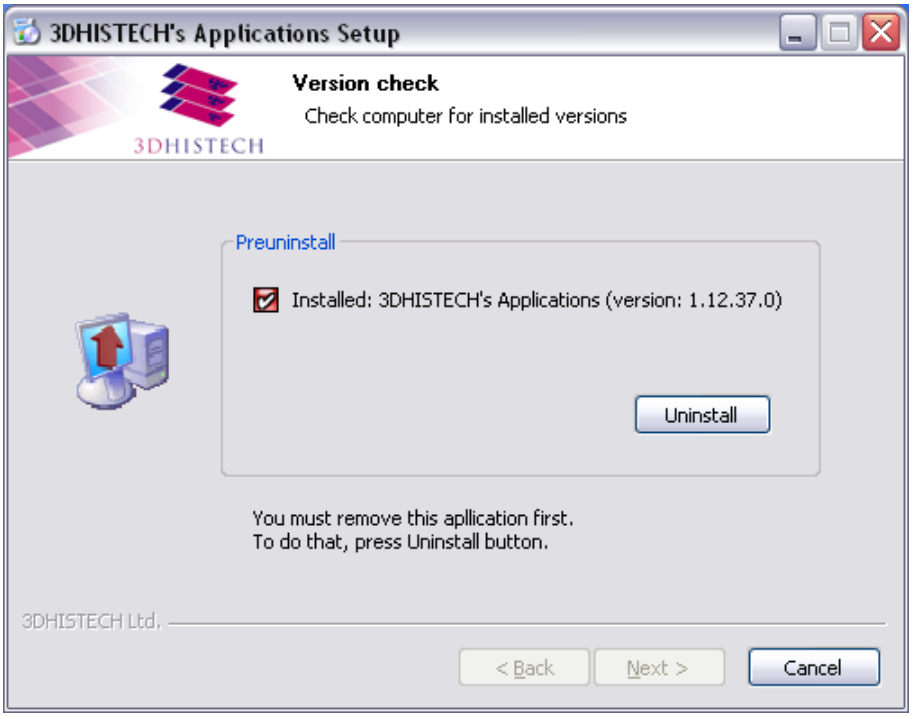


A progress bar and an information box is displayed, showing the status of the installation procedure.



9. After completing the installation of MIRAX Viewer and its modules, click **Finish** to close the wizard.

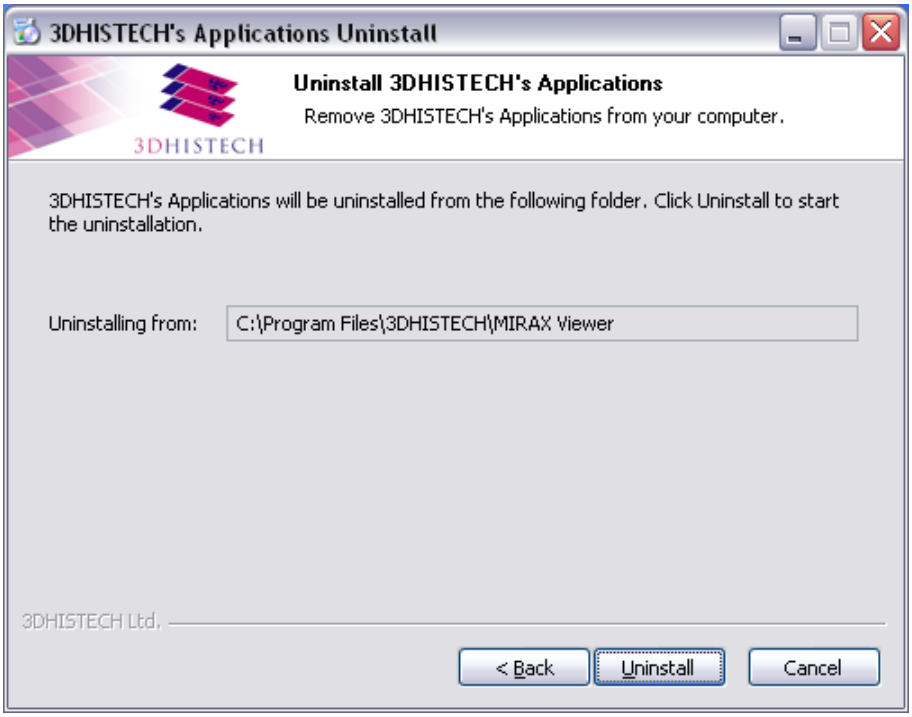
10. If you have an older or newer version of the applications, you have to uninstall them first.



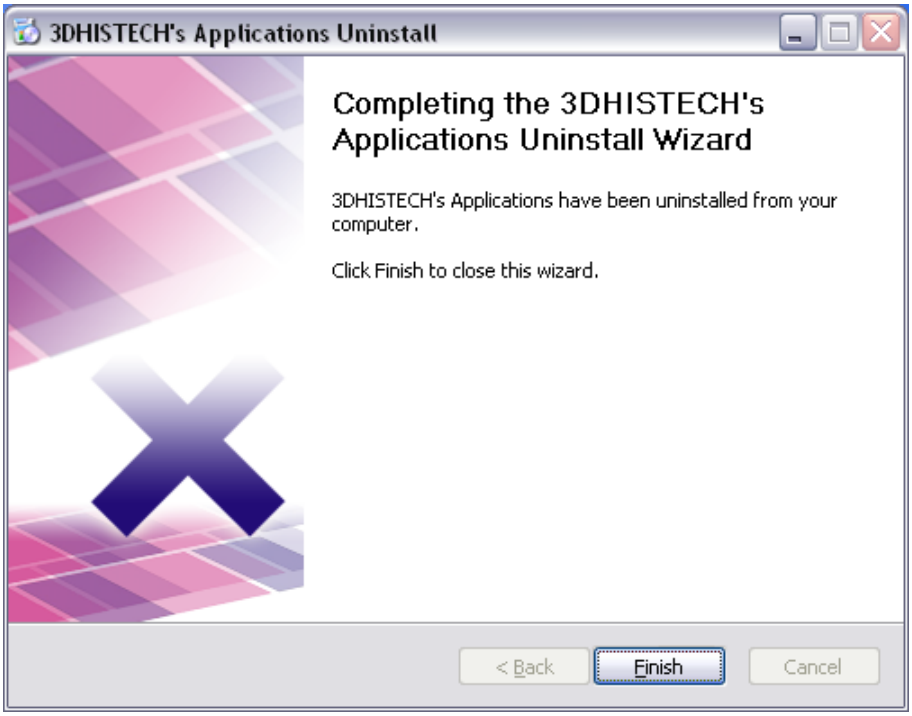
11. If the uninstall wizard window opens, click **Next**.

3DHISTECH's Applications Uninstall
Welcome to the 3DHISTECH's Applications Uninstall Wizard
This wizard will guide you through the uninstallation of 3DHISTECH's Applications.
Before starting the uninstallation, make sure 3DHISTECH's Applications are not running.
Click Next to continue.
Next >
Cancel

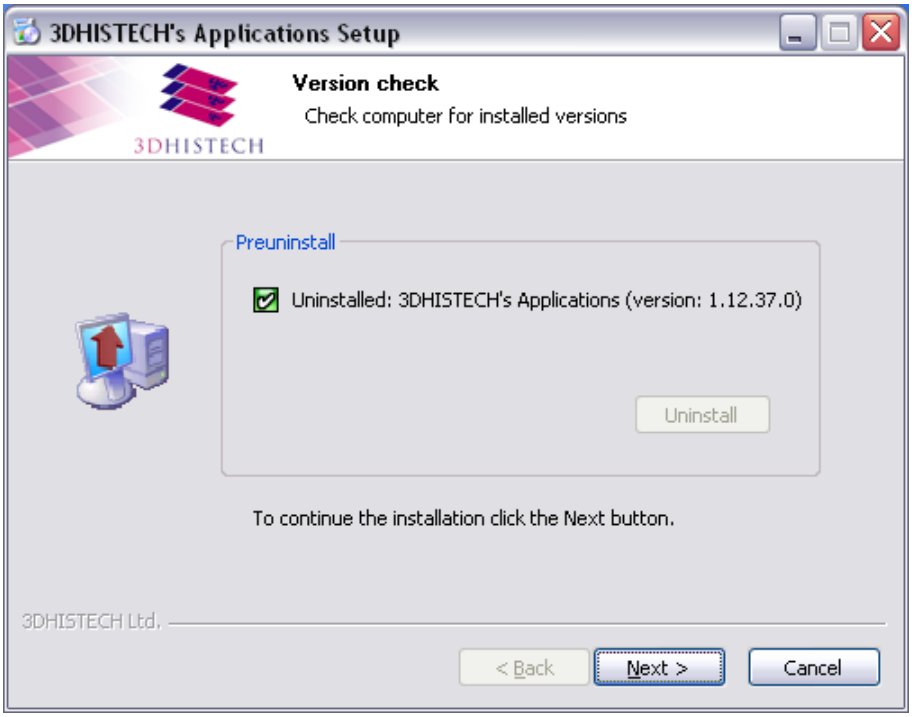
12. The wizard automatically detects the folder of the previously installed applications. Click **Uninstall** to proceed.



13. When the uninstallation of MIRAX Viewer and its modules is complete, click **Finish** to close the wizard.



14. After the previously installed applications are removed, click **Next** to launch the installation wizard.



3 Working with MembraneQuant

3.1 Before you start

MembraneQuant works on selected *annotation*s. You can run other algorithms on several *annotation*s by selecting all the *annotation*s that you need when you start the algorithm.

Important!

MembraneQuant algorithms utilize the computer's hardware (mainly the CPU and the memory). The speed of the **MembraneQuant** evaluation depends on the hardware configuration and performance, the size and the number of the measured *annotation*s, and the quantity of detected *objects*.

MembraneQuant algorithm runs only on Brightfield slides.

To ensure accurate results and to reduce computation time:

- Make the training *annotation* as small as possible. The annotated areas should not be larger than 300x300 µm (1:1 magnification).
- When you are creating a free-hand *annotation*, follow the outline of the area as closely as possible.
- Select the *annotation* area appropriately, for example, select an area that is rich in tumor cells instead of connective tissue.

Tip!

If you need to make *measurement*s on the entire specimen, simply select the whole slide.

3.2 Creating Annotation for MembraneQuant

1. Open the slide in MIRAX Viewer. For more information on how to open slides in MIRAX Viewer, see section **Working with slides in MIRAX Viewer** in the *MIRAX Viewer 1.12 User's Guide*.

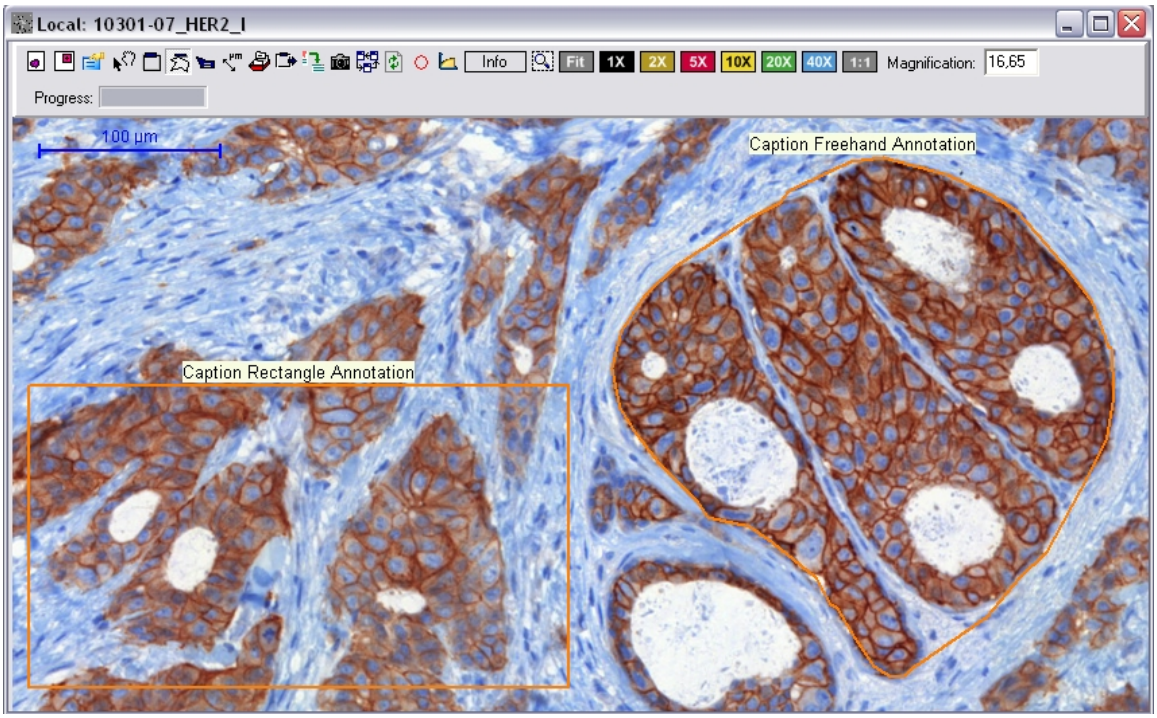
2. Create the *annotation* on the area of your interest:

- Select the *annotation* type (rectangle or free-hand) and cover the area with the *annotation*.

  For information on navigating and zooming on the slide, see section **Viewing slides** in the *MIRAX Viewer 1.12 User's Manual*.

- In the **Create or Edit Annotation** window add a title to the *annotation*, enter a comment and determine the background color of the title and the outline color of the *annotation*. You can either select from predetermined colors or you can define custom colors.

  Only captions are exported to the *CSV* output file. *Annotation* descriptions are visible only in MIRAX Viewer.



3. Repeat step 2 for each area on which you want to run the algorithm.

3.3 Running the Algorithm on a Single Slide

Open the slide in MIRAX Viewer.

1. In the main toolbar, click the icon and select **MembraneQuant** from the **Image Analysis Modules**.

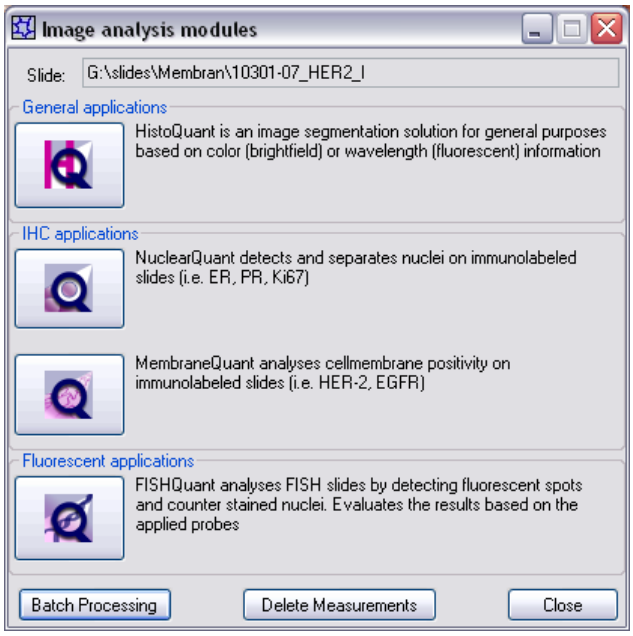
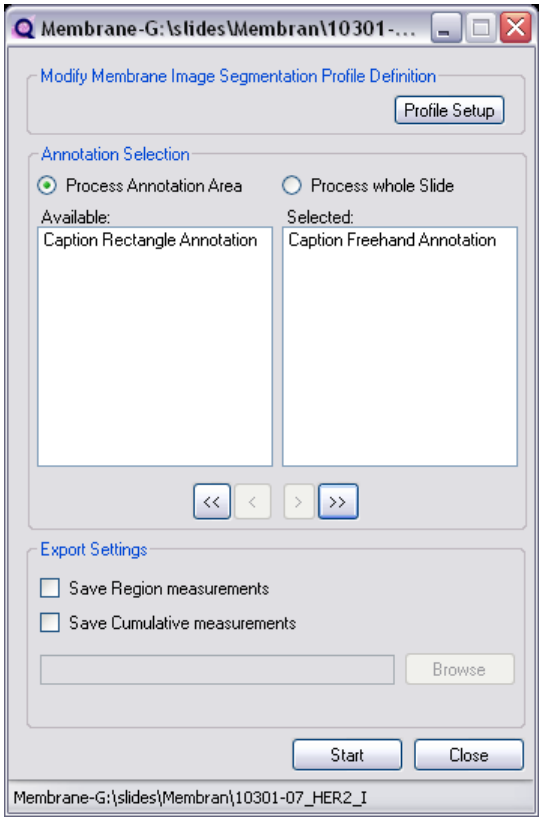


Image analysis modules
Slide:
G:\slides\Membran\10301-07_HER2_I
General applications
HistoQuant is an image segmentation solution for general purposes based on color (brightfield) or wavelength (fluorescent) information
IHC applications
NuclearQuant detects and separates nuclei on immunolabeled slides (i.e. ER, PR, Ki67)
MembraneQuant analyses cellmembrane positivity on immunolabeled slides (i.e. HER-2, EGFR)
Fluorescent applications
FISHQuant analyses FISH slides by detecting fluorescent spots and counter stained nuclei. Evaluates the results based on the applied probes
Batch Processing
Delete Measurements
Close

2. Select the *annotation* or *annotation*s you want to analyze, in the **Available** window at the **Annotations Selection** panel, then move them to the **Selected** window by clicking **>** or **>>**.

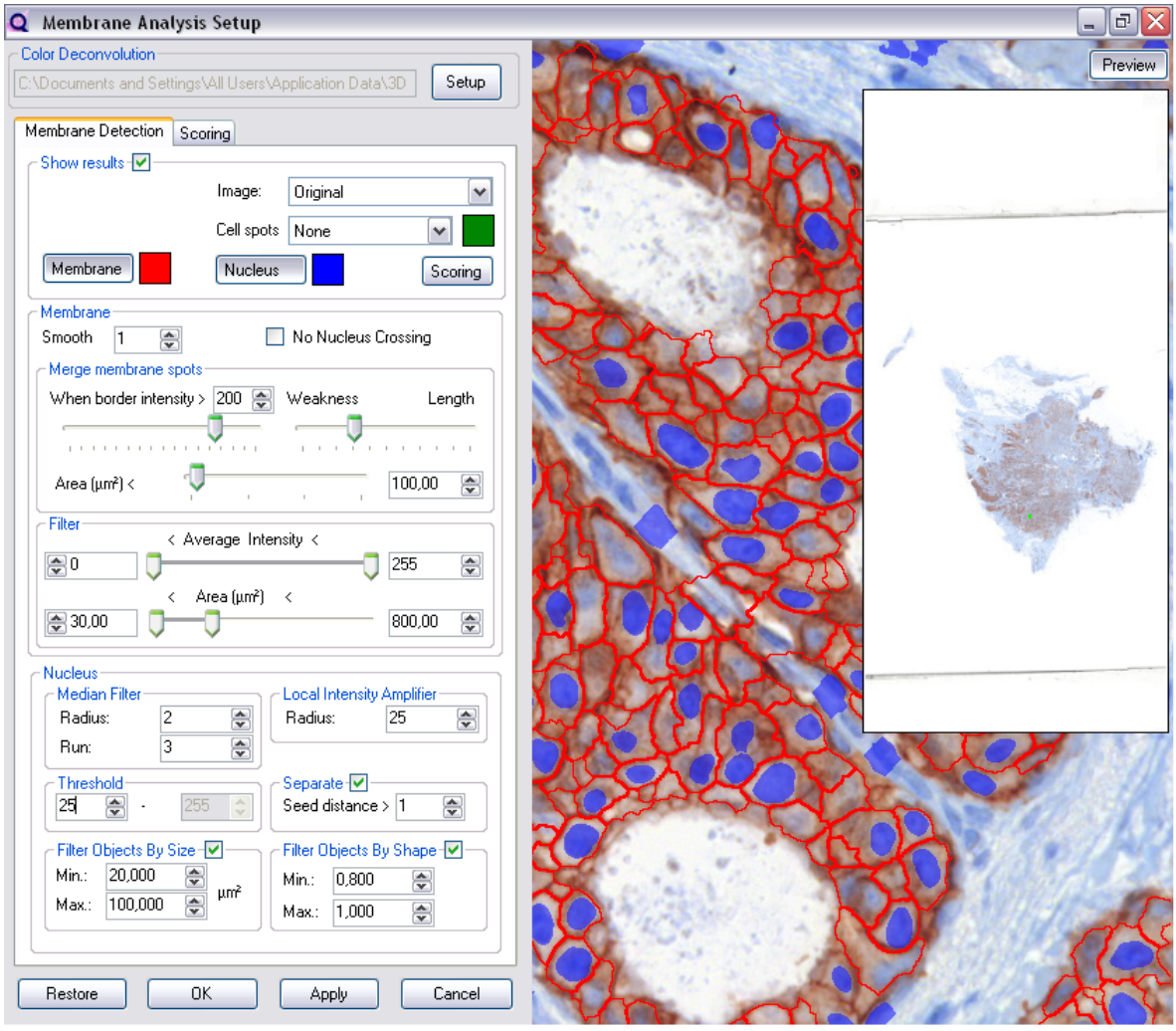
   If you need the whole slide, check **Process whole Slide**.



Profile Setup	Use this application to set or change the **Profile Setup** of the

	MembraneQuant algorithm.
Annotation Selection	You can select your *annotation*(s) for the measuring process.
Process Annotation Area	The algorithm will be executed only on the selected *annotation*s.
Process Whole Slide	The *annotation* will be executed on the whole *digital slide* area.
Export settings	**Save Region measurements**: The *measurement*s will be exported to a *CSV* file that you can open and edit in any spreadsheet software (for example. MS Excel, OpenOffice). **Save Cumulative measurements**: The *measurement*s will be exported to *CSV* files. A cumulative file will be created for each detected *layer*. **Save to single CSV**: The *measurement*s will be exported to a single *CSV* file. This file will contain all the parameters of the *layer*s.

3. Select **Profile Setup** to define the parameters of the algorithm. The Membrane Analysis Setup window opens.



4. Before setting the parameters of the detection in the **Membrane Analysis Setup** window, create a Color Deconvolution Profile.

3.3.1 Setting up a Color Deconvolution Profile

5. Click **Setup** to specify Color Deconvolution parameters.

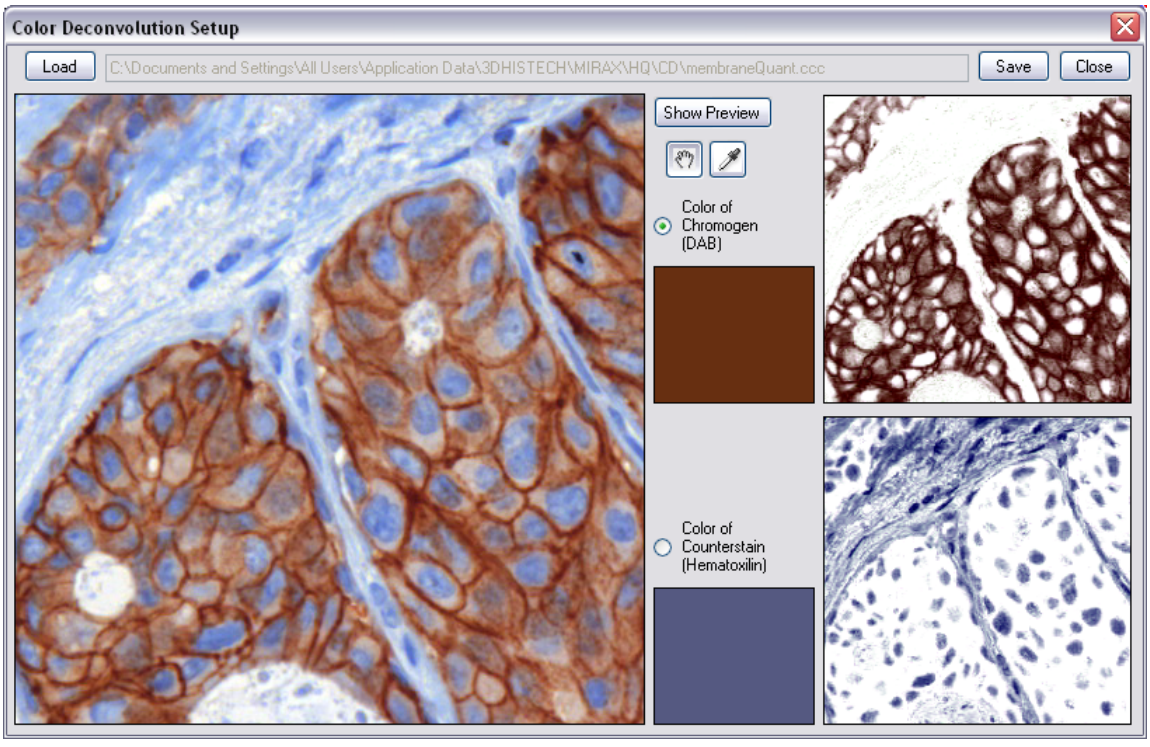
> **Important!**
>
> There are four preset *CCC* files according to different camera sensitivities.

You can set the software to your automated slide stainer or labor protocol with this application. When assigning manual settings, it is recommended to take random intensity samples from the preview screen of the most dense areas of the chromogen.

If you get the desired result in the chromogen preview and the parameters are set correctly, modify the color settings of counterstain. Marking with chromogen can result in a strong background color; please, take this into account when sampling counterstain. It is recommended to take samples from the background area of the image.

Note: The software automatically refreshes the displayed chromogen and counterstain preview images according to your settings.

In the **Color Deconvolution Setup** window, you see a magnified image of the slide where a preview of the original can be switched on and off by pressing **Show Preview**, and the RGB color spectrum of the marker colors can be adjusted.

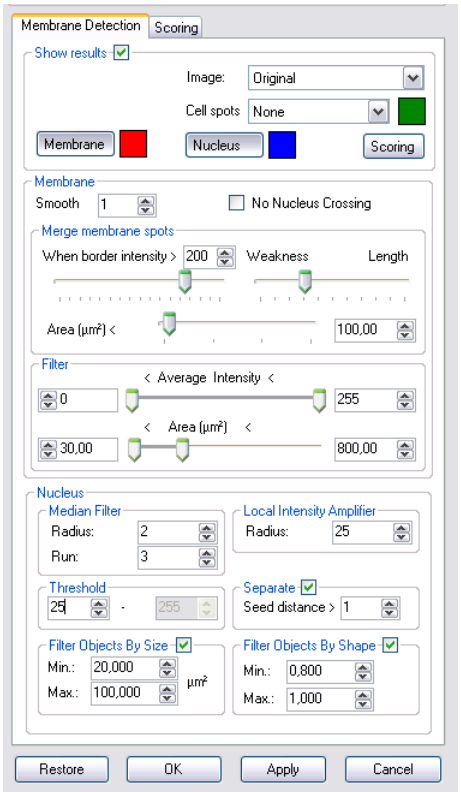


Load	You can load predefined Color Deconvolution Profiles (*CCC* files).
Save	You can save your settings to Color Deconvolution Profiles.
Show Preview	A preview window opens where you can select the area of your interest.
	You can position the image in the left-side window by dragging.
	You can get color samples from the image on the left.
Color boxes	Left-click on the boxes to adjust the RGB color spectrum if necessary, with the help of the slides or type in the required values (press **Enter** to refresh). Right-click on the boxes to reset the sampled color to black.

Note: The software continuously refreshes the two preview images on the right side of the window during sampling, and also note, that sampling has effect on both preview images.

3.3.2 Setting Detection Parameters

6. After saving a Color Deconvolution Profile, click **Close**, and define the parameters of the algorithm in the **Membrane Detection** tab on the left side of the **Settings Preview** window.



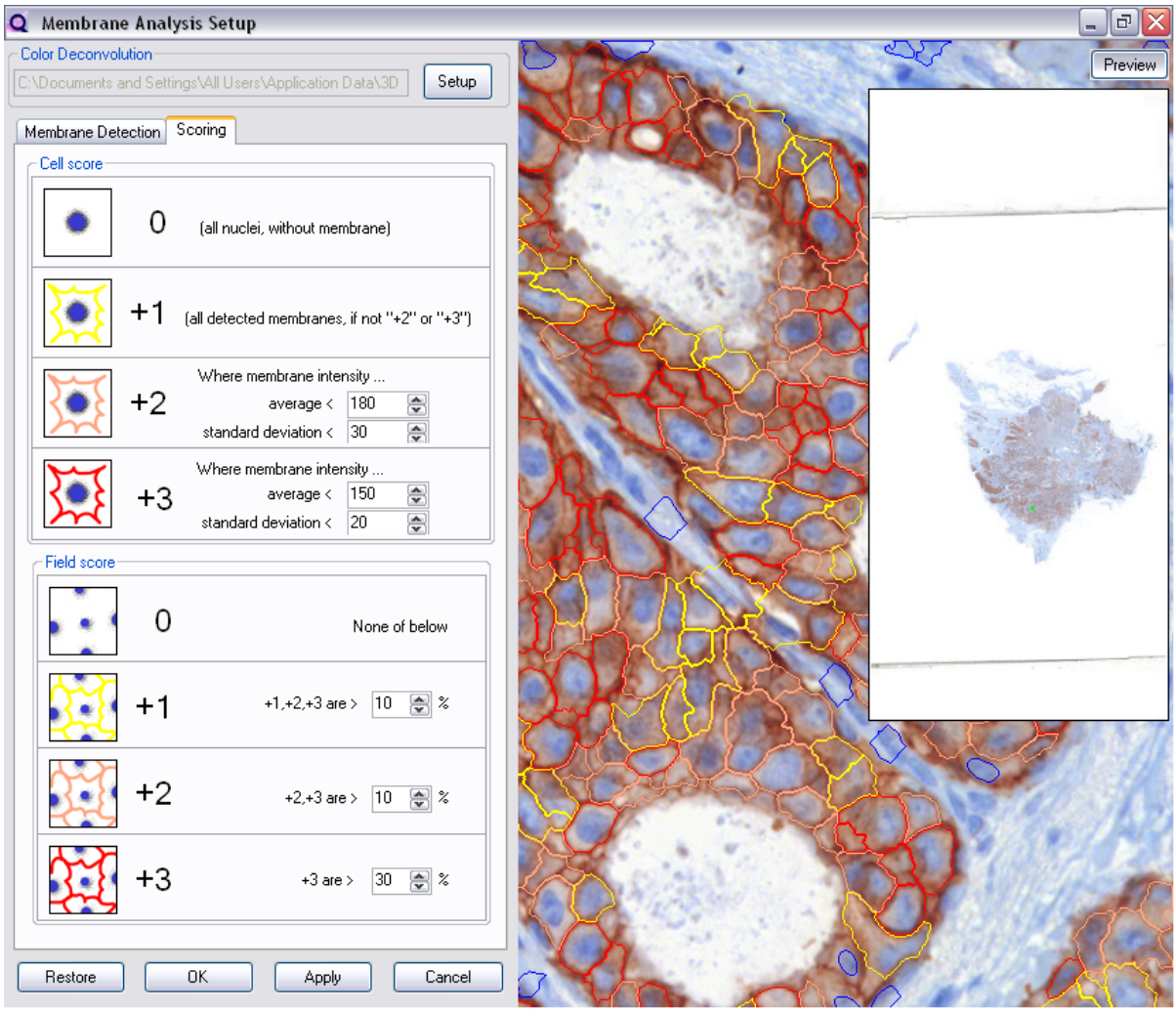
Color Deconvolution	Use this to set up the CD (Color Deconvolution) parameters.
Image	Shows the mask and various aspects of the digitized slide.
Membrane	Shows the various aspects of the membrane algorithm. **Smooth**: Blurring is recommended on an ordinary histology sample (smoothness value is 3 by default), and when working on low quality samples (too much noise on the image) it is recommended to raise the value. **Merge membrane spots:** Membranes can be merged respectively to their border intensity, weakness-length, and area. Change parameters until you get the desired detection result in the preview window.
Membrane Settings	Parameter settings of the membrane algorithm. You can adjust the smoothness and the merging values.
Filter	Sets the size in µm and the average intensity of the *object*s that **MembraneQuant** should detect as *object*s. There is a post-processing filter option in the algorithm based on Average intensity and Area values. Thus, the superfluous tissues, gland lumens, and cell cavities can be excluded from the detection process.
Nucleus Settings	Parameter settings of the nucleus. You can adjust the threshold, the smoothness and the erosion values. You can also filter *object*s by size and shape.
Median filter	Simple, but fast filtering method; it uses the median of adjacent pixel values. A 3X3 blurring is recommended on an ordinary histology sample, and when working on low quality samples (too much noise on the image) it is recommended to raise up the values.
Local Intensity Amplifier	Amplificates the local maximums, the area of which complies with the R radius filter. Sets the intensity to the average cell size. The value is 20-25 pixels on a sample taken with 20x magnification level/zoom.
Threshold	Sets the pixel intensity threshold (values under the threshold will be defined as background). The optimal value is between 20 and 30 on an average intensity slide.
Separate	The pixel distance between two seeds which separates the spots if the given value is smaller than half of the seed-distance. The less the value, the more it reduces the distance.
Filter Objects By Size	Sets the size of the *object*s in μm^2 that **MembraneQuant** should detect as *object*s. Excludes pixel groups that are too small or too large. It enables you to filter the smaller nuclei of the inflamed part of the tissue.
Filter Objects By Shape	Sets the shape of the *object*s that **MembraneQuant** should detect as *object*s. This filtering method marks the roundish nuclei of the connective tissue cells distinguished from the epidermal cells that have high shape factor. Value ranges from 0-1, where 1 marks the round nuclei.

3.3.3 How to set the Scoring

7. Select **Scoring** tab and set the membrane scoring setup.

8. In the **Scoring** tab, you can set Average Intensity values for the nuclei marked with +1, +2, and +3.

   Automatic scoring can be set based on pixel intensity values. When defining scoring values, please, take the previous settings and results of Color Deconvolution into account.

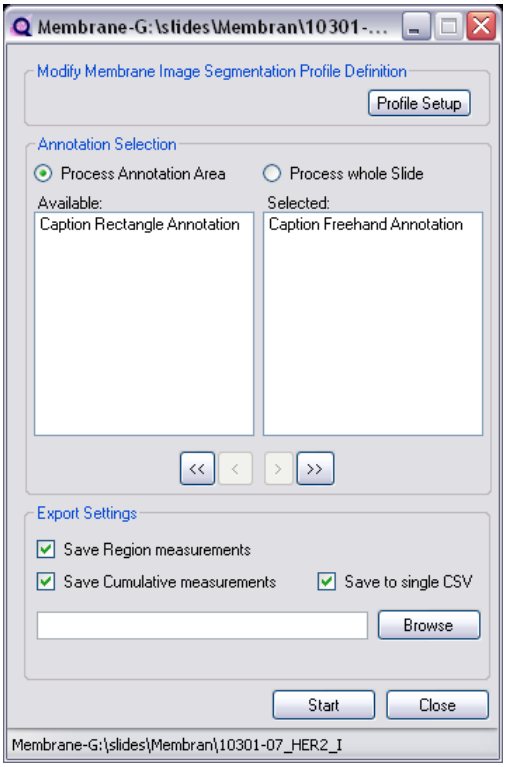


Cell score	Scores the cell membranes based on their intensity.
Field score	Scores the whole *field*.
Average membrane intensity	Scores an area based on the average intensity.
Std. Dev. of membrane intensity	Indicates the allowed deviation in intensity.

Up, down, left, right	Moves the *annotation* up, down, left and right.

9. Save your settings and close the **Membrane Scoring Setup** form by clicking **Ok**.

10. [optional] You can export Membrane Analysis *measurements* as *CSV* files by specifying the **Export Settings.** Specify where the *CSV* file(s) should be saved.



Save Region measurements	The *measurements* will be exported to a *CSV* file that you can open and further edit in any spreadsheet software (for example, MS Excel, OpenOffice).
Save Cumulative measurements	Cumulative *measurements* will be exported to *CSV* files. A cumulative file will be created for each detected *layer*.
Save to single CSV	The *measurements* will be exported to a single *CSV* file. This file will contain all the parameters of the *layers*.

11. Launch the *MembraneQuant algorithm* by clicking **Start** in the **Membrane** window.

Warning!

By using the same *annotation* and *MISP* file, the program overwrites the previously saved *CSV* file. If you want to create a different file, locate an-

other folder to save *measurement* data.

The process is fully automated, you only need to click **Close** when **MembraneQuant** has finished. The application leaves the slide open. If more than one slide is open when you launch the Wizard, **MembraneQuant** always uses the active one.

Tip!

To view the existing *annotation*s and *measurement*s on the slide, click **INFO** in the slide toolbar and then select **Show Annotations** or **Show HQ Measurements** from the shortcut menu.

3.4 Running Batch Process in MembraneQuant

In **Image Analysis Modules** menu, you have the option to work in batch mode. Select the slides you want **MembraneQuant** to work with and specify the parameters according to which they should be analyzed.

3.5 Saving MembraneQuant Results to the Slide

MembraneQuant automatically saves the following data results to the slide:

- outline of the detected *object*s
- measured data: morphometrical or colorimetrical data on the detected *object*s and area-specific parameters

3.6 Exporting MembraneQuant Results in CSV Format (Measurements from One Slide)

When you create the *MISP* file, **MembraneQuant** exports the results to a *CSV* file. If you need *measurement*s from this slide, provide a new file name.

If you create several *layer*s in the *annotation* with the *MembraneQuant algorithm*, the data for each *layer* can be exported into a separate *CSV* file. Furthermore, a cumulative *CSV* file is also exported with the *layer* data.

Note: CSV file names are generated automatically from the combination of names of *MISP*, slide, and *annotation*.

1	Slide information
2	Name of the *annotation*(s) that you selected for the *CSV* export If you selected several *annotation*s on the same slide for the *CSV* export, the *CSV* file lists them at the top of the table; the *object* IDs of the *annotation*s are listed one after the other at the bottom section of the table.
3	*Object*s Each *object* that the *MembraneQuant algorithm* found in the *annotation* is shown in a separate row.
4	Parameters Each parameter is shown in a separate column.
5	*Measurement* Each *measurement* (the value of a parameter in an *object*) is shown in a separate cell.

3.7 Exporting MembraneQuant Results to a Cumulative CSV File

When you create the *MISP* file, **MembraneQuant** exports the results to a *CSV* file. If you require *measurement*s from several slides to create a cumulative database, select the *CSV* file to which **MembraneQuant** appends two rows.

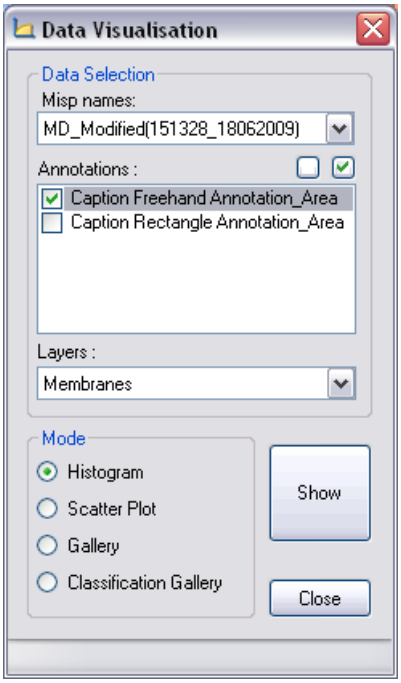
1	Slide information
2	The *layer* information is visible in the parameter names in the column headers.
3	Parameters that you selected for the *MembraneQuant algorithm* and for which *measurement* data is available for *CSV* export.

4 Working with Measurement Visualization

The **Measurement Visualization** module for MIRAX Viewer creates a scatter plot or a histogram from the **MembraneQuant** *measurement*s.

4.1 Creating a Histogram

1. Click the icon in the slide toolbar in the MIRAX Viewer.



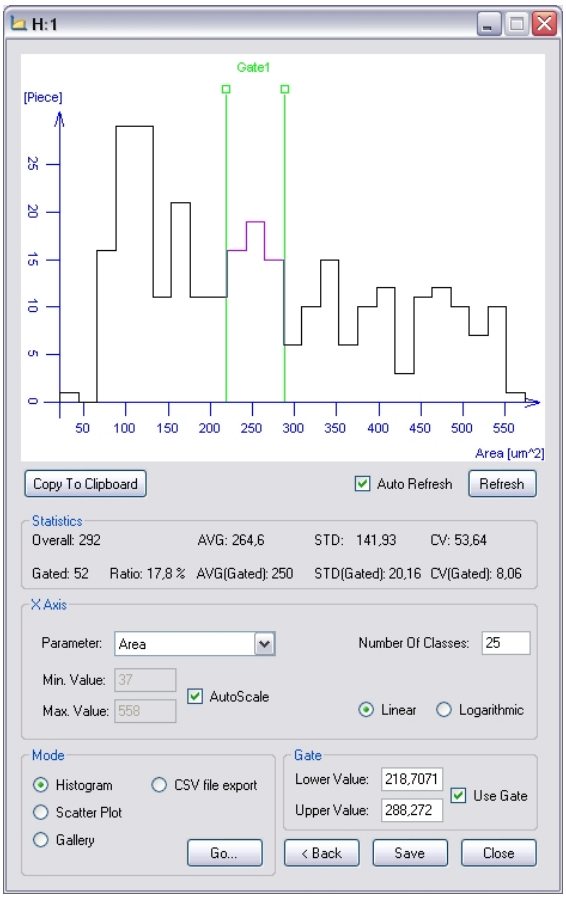
2. Select the name of *measurement* (previously saved to the slide) from the **MISP names** drop-down menu in the **Data Selection** panel.

3. Select the *annotation*s you want to display. By clicking the right-side checkbox above the **Annotations** window, you can select all the *annotation*s. To remove selections, click the checkbox to the left.

4. Select the *object layer* that you want to display from the **Layer** drop-down menu.

5. Select **Histogram** and the press **Show** button**.**

6. Set the parameters. If you enter new values into the text *field*s, press **Enter** to refresh the diagram.



Auto Refresh	Automatically updates the histogram when you change the settings for the parent form.
Statistics	Overall shows the quantitative data of the *objects*.
X Axis	Offers various parameters for the X axis. When you export the results into a *CSV* file, there is an explanation for the abbreviations of the parameter names.
Gate	Chooses an interval within the examined range on X axis.
Mode	By using Gate function, the program either creates new histogram, new scatter plot, shows gallery or opens a new form where the user can save *CSV* files, but only for the selected interval. By not using Gate function, the program uses all the above mentioned features but for the whole data range.

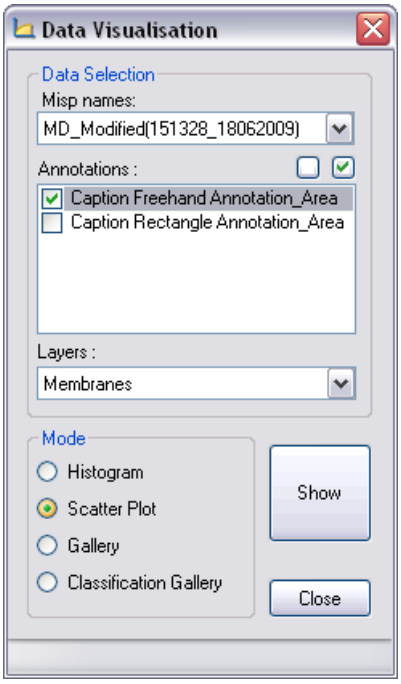
7. Press **Back** to get to the parent window.

8. To create a snapshot of the histogram, click **Save**.

9. To exit Measurement Visualization, click **Close**.

4.2 Creating a Scatter Plot

1. Click the icon in the slide toolbar in the MIRAX Viewer, only if no other data visualization window is open.



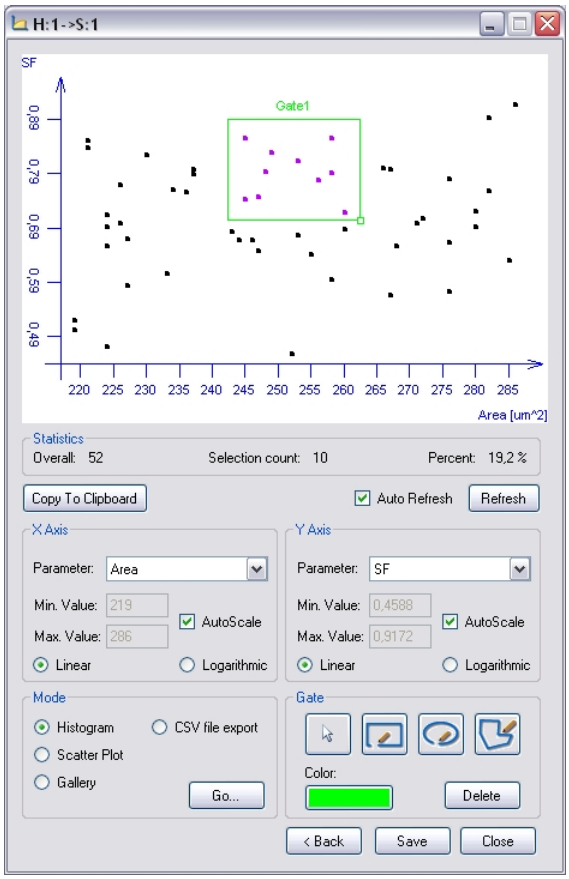
2. Select the name of *measurement* (previously saved to the slide) from the **MISP names** drop-down menu in the **Data Selection** panel.

3. Select the *annotation*s that you want to display.

4. Select the *object layer* that you want to display.

5. Select **Scatter Plot** and the press the **Show** button.

If you select **Scatter Plot** button in the **Mode** panel of the previously opened **Data Visualization** window, then press **Go...** . The process steps can be seen in the title bar of the new window, for example, H:1 → S:1.



6. Set the parameters. If you enter new values into the text *field*s, press **Enter** to refresh the diagram.

Auto Refresh	Automatically updates the histogram when you change the settings for the parent form.
Statistics	Overall it shows the quantitative data of the *object*s.
X Axis	Offers various parameters for the X axis.
Y Axis	Offers various parameters for the Y axis.
Gate	Chooses an interval within the examined range on X axis. You can position the scatter plot gates by clicking on the arrow.
Mode	By using Gate function, the program either creates new histogram, new scatter plot, shows gallery or opens a new form where the user can save *CSV* files, but only in the selected interval. By not using Gate function, the program uses all the above mentioned features, but for the whole data range.

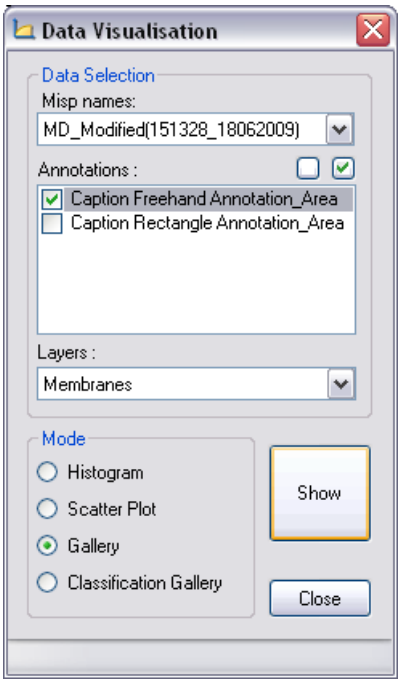
7. Press **Back** to get to the parent window.

8. To create a snapshot of the histogram, click **Save**.

9. To exit Measurement Visualization, click **Close**.

4.3 Creating a Gallery

1. Click the icon in the slide toolbar in MIRAX Viewer, only in case no other data visualization window is open.



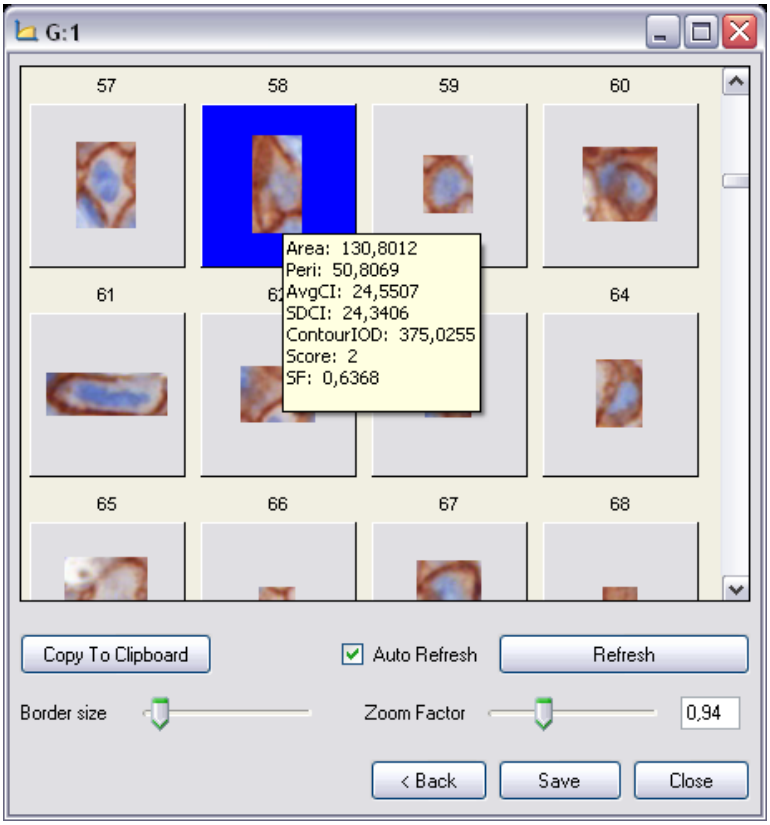
2. Select the name of *measurement* from the **MISP names** drop-down menu in the **Data Selection** panel.

3. Select the *annotation*s that you want to display.

4. Select the *object layer* that you want to display.

5. Select **Gallery** and the press the **Show** button**.**

If you select **Gallery** button in the **Mode function** panel of the previously opened **Data Visualization** window, then press **Go...**. The process steps can be seen in the title bar of the new window, for example, H:1 → S:1 → G:1.



Auto Refresh	Automatically updates the histogram when you change the settings for the parent form.
Border size	Sets the size of the area around the object.
Zoom factor	Sets the zoom rate of the images. You can determine the zoom value by entering the exact rate in the text *field*.

If you move the cursor over an image an info box appears conveying all the necessary data regarding that image.

6. Press **Back** to get to the parent window.

7. To export the gallery as an image, click **Save**.

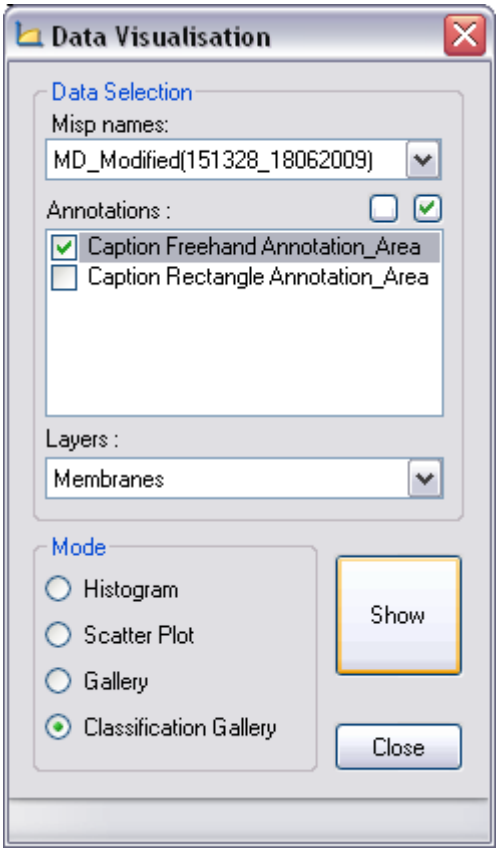
8. To exit Measurement Visualization, click **Close**.

Tip!

If you select an image in the gallery, it will be highlighted in the scatter plot as well.

4.4 Creating a Classification Gallery

1. Click the icon in the slide toolbar in MIRAX Viewer, only in case no other data visualization window is open.



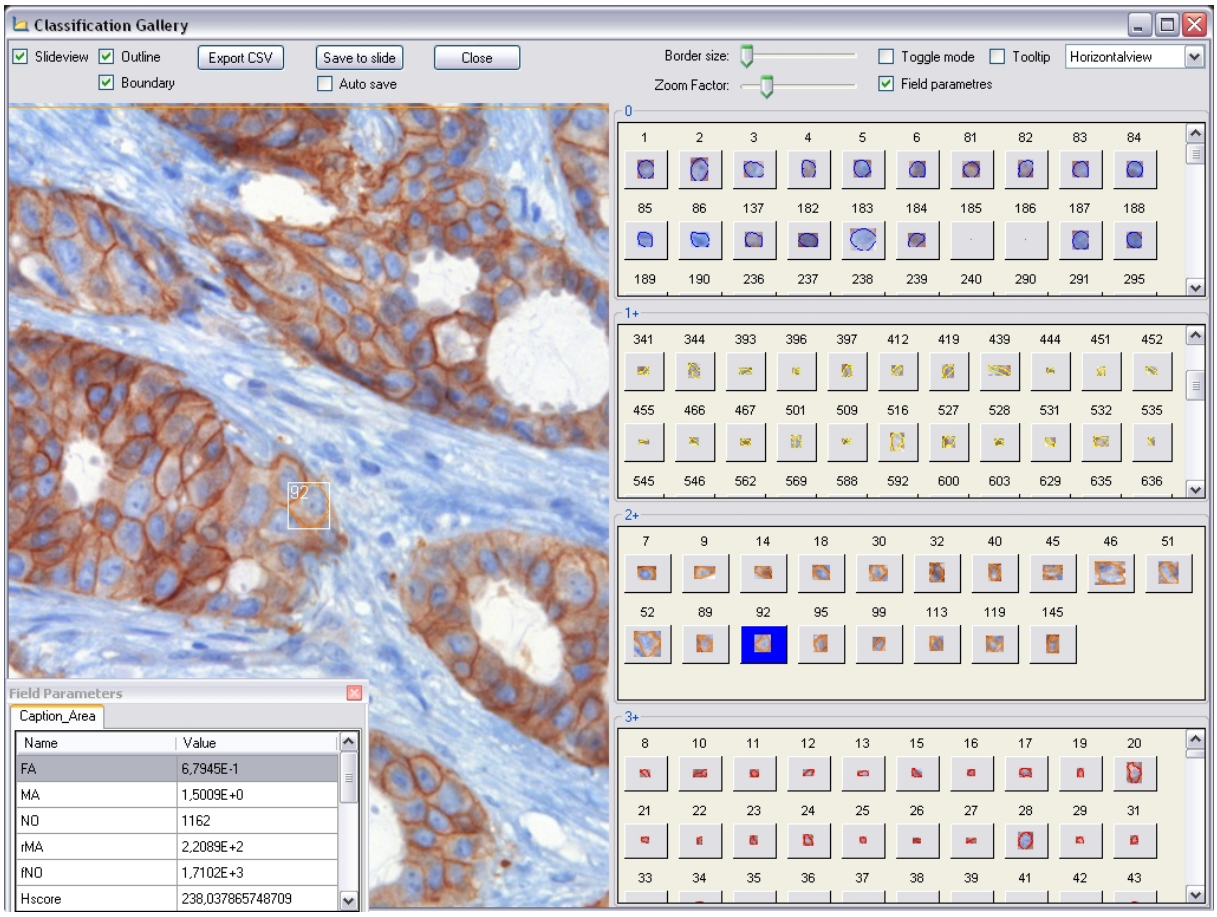
2. Select the name of *measurement* from the **MISP names** drop-down menu in the **Data Selection** panel.

3. Select the *annotation*s that you want to display.

4. Select the *object layer* that you want to display.

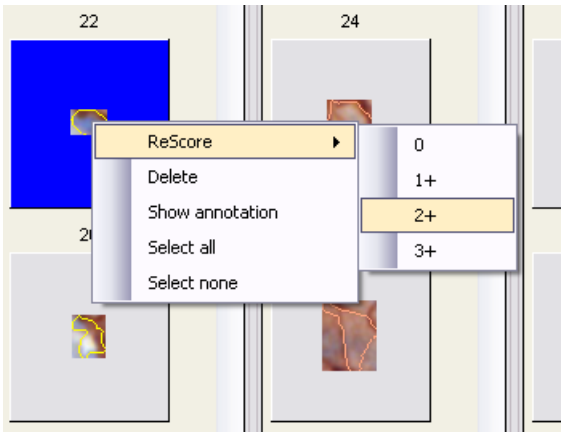
5. Select **Gallery** and the press the **Show** button**.**

You can group your *object*s in accordance with their score values. You can revalidate and rescore your *object*. On the right side panel, you can move cell nuclei from one group to the other. On the left panel, you can view the selected cell nuclei.

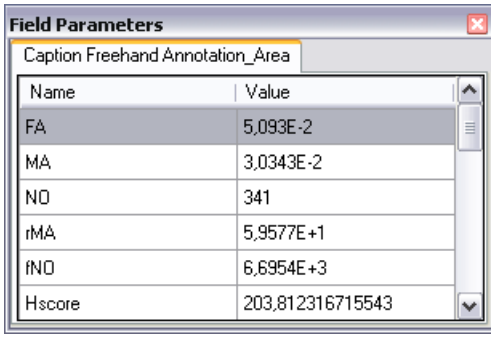


Slideview	Shows the selected slide.
Outline	Shows the group-specific outline.
Boundary	Shows the number of the selected *objects*.
Export CSV	Exports the *measurements* to a *CSV* file.
Save to slide	Saves the changes to the slides.
Auto save	Automatically saves all changes. Note, that it decelerates the program.
Border size slider	Increases / decreases the size of *object* borders in the right panel.
Zoom factor slider	Zooms in / out on the *objects* in the right panel.
Toggle mode	You can select *objects* with the help of your mouse in the right panel. You can rescore *objects* using right-click.
Tooltip	Moving cursor over an *object* shows the basic information of the given *object*.
View	You can select among normal, horizontal or vertical view of the *objects* in the right panel.

By right-clicking on a selected *object* or selected *objects*, you can rescore or delete them, select all or none, and/or show their *annotations*.



The **Field Parameters** window lists parameter names and values of that specific *annotation*. Scroll down or change window size to see the whole list.



Related documents

MIRAX Viewer 1.12 User's Guide

ImmunoScreener Module for MIRAX Viewer 1.12, User's Guide

MIRAX SCAN User's Guide

Image processing and analysis
(http://books.google.com/books?id=kvWKgG6iPXMC&dqÖ)

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Appendix A - MembraneQuant

A.1 Object-specific parameters

Parameter name	Description
Area	Area of the detected *object* in µm^2
BInt	B intensity of the detected *object*
GInt	G intensity of the detected *object*
GrayInt	Gray intensity of the detected *object*
IF_C1	Integrated fluorescence in channel 1
IF_C2	Integrated fluorescence in channel 2
IF_C3	Integrated fluorescence in channel 3
LDia	Longest diameter of the detected *object* in µm
Peri	Perimeter of the detected *object* in µm
RInt	R intensity of the detected *object*
SDi	Shortest diameter of the detected *object* in µm
SF	Shape factor of the detected *object*

A.2 Summing parameters for an area

Parameter name	Description
FA	Overall *annotation* area in mm^2
fNO	NO/FA; frequency of detected *object*s per µm^2
HSCORE	1*('NO+1'/'NO')*100 + 2*('NO+2'/'NO')*100 + 3*('NO+3'/'NO')*100
MA	Overall mask area in mm^2: Total area of all detected *object*s in each *layer*.
MA0	Mask area of negative Membrane
MA+1	Mask area of +1 Membrane
MA+2	Mask area of +2 Membrane
MA+3	Mask area of +3 Membrane
NO	Number of detected *object*s: Total number of each detected *object* in each *layer*
NO0	Number of negative Membrane
NO+1	Number of +1 Membrane
NO+2	Number of +2 Membrane
NO+3	Number of +3 Membrane
rMA	(MA/FA)*100; relative mask (*object*) area in %
rMAPos	(('MAO+1' + 'MAO+2' + 'MA+3') / MA)*100
rNOPos	(('NO+1' + 'NO+2' + 'NO+3') / NO)*100